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CHROMOSOME POLYMORPHISM IN *GOBIUS PAGANELLUS*, LINNEO 1758 (PISCES, GOBIIDAE)

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Abstract

In the present investigation the diploid numbers 2n = 45, 2n = 46, 2n = 47, and 2n = 48 have been determined for *Gobius paganellus.* Specimens of different sex were found to have exactly the same karyotype. This species is characterized by two fundamental numbers: NF = 47, and NF = 48. Chromosome polymorphism due to different chromosome rearrangements within the A-type complement is present in this species.

Introduction

The family Gobiidae is quite interesting because of its controversial morphological features (Fage, 1925; Arai and Sawada, 1974; Nishikawa *et al.,* 1974) and its evolutionary stage which is not completely known (Chen and Ebeling, 1971; Chiarelli and Capanna, 1973; Arai *et al.,* 1974; Manna and Prasad, 1974; Arai and Sawada, 1975; Khuda-Bukhsh, 1978; Colombera and Rasotto, 1982). A high variability of chromosome number occurs within this group ranging from 2n = 40 to 2n = 62 (Sola *et al.,* 1979).

There still is no agreement as to the number of chromosomes characterizing *Gobius paganellus.* The diploid number 2n = 45 was proposed for a female specimen caught in the Thyrrhenian Sea (Cataudella *et al.,* 1973), n = 25 and 2n = 50 for male specimens caught in the Northern Adriatic Sea (Colombera and Rasotto, 1982), and 2n = 46 for eight male and female specimens caught in the Southern Mediterranean Sea near Spanish coasts (Thode *et al.,* 1983).

Thode *et al.,* (1983) claim that *Gobius paganellus* is characterized by male heterogamety (XY). The occurrence of a large metacentric chromosome in the female specimen investigated by Cataudella *et al.* (1973) does not agree with the mechanism of sex determination proposed for this species.

The present investigation was aimed at clarifying these problems by analyzing chromosome sets from numerous male and female *Gobius paganellus,* Linneo 1758, specimens.

Materials and Methods

Twenty-six *Gobius paganellus* specimens (17 females, 7 males, and 2 sexually immature specimens) caught in the Gulf of Palermo were analyzed and classified according to the guidelines of Tortonese (1975) and Bini (1969). Specimens from this study were deposited at the Institute of Zoology of the University of Palermo.

Each specimen was injected intraperitoneally with colchicine (0.1%, 1 ml/30 g body weight) and sacrificed two hours later. Kidney and spleen tissues were removed, and minced in 0.075 *M* KCl. The suspension was centrifuged for twenty minutes

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and the supernatant was discarded, while cell sediment was fixed in two successive changes of fresh methanol-acetic acid (3:1). Drops of cell suspension were then placed on clean slides at 0°C. Slides were prepared by air-drying and stained with 2.5% Giemsa (Gurr, R 66) for 20–30 minutes.

Observations and photographs were made using a Wild or Leitz microscope. Chromosomes were classified according to arm ratio, as proposed by Levan *et al.* (1964). Metacentric (M) chromosomes are referred to as bi-armed chromosomes, while sub-telocentric (ST) and acrocentric (A) chromosomes are referred to as mono-armed chromosomes.

Results

Observations were made on six female specimens and one sexually immature specimen with the modal chromosome number 2n = 48 (Table I). An average karyotype, arbitrarily designated as A-type complement (Fig. 3; Table II), was obtained by measuring and arranging pairs of homologous chromosomes from nine metaphase plates by decreasing length and according to centromere position. These chromosomes cannot be grouped into classes according to length, since their size ranges from 2.67 to 0.84 μm and they are all acrocentrics (Fig. 1a, b) except for one element of the first pair (Fig. 2a, b) which was observed in five specimens, in which AR = 3.13. This chromosome can therefore be classified as sub-telocentric.

Table I

Diploid chromosome counts in 26 specimens of Gobius paganellus

Specimen number	Sex	Number cells analyzed	Diploid counts ≤40	41	42	43	44	45	46	47	48	49	≥50
1	♀	18							2	1	15		
2	♀	28	3			1		1			22	1	
3	♀	33	2			1			2		28		
4	♀	30					1	2			25		2
5	♀	30		2					2	1	25		
6	♀	27					2			2	23		
7	?	18					1			2	15		
8	♀	37	3					3	1	30			
9	♂	16	1							15			
10	?	32	3	2						27			
11	♂	27					1		2	23	1		
12	♂	12	1					1		10			
13	♀	13		2				1		10			
14	♀	23				2			1	20			
15	♂	36						2	2	32			
16	♀	28	2					1	3	22			
17	♀	26	4					2		20			
18	♂	30				2		3		25			
19	♂	17		2			1		15				
20	♀	39	2				1	1	35				
21	♀	20	1				1		18				
22	♀	23		1				2	20				
23	♀	20						1	18	1			
24	♀	18	2				1		15				
25	♂	30	2	1			2	25					
26	♀	48	3			3	2	40					
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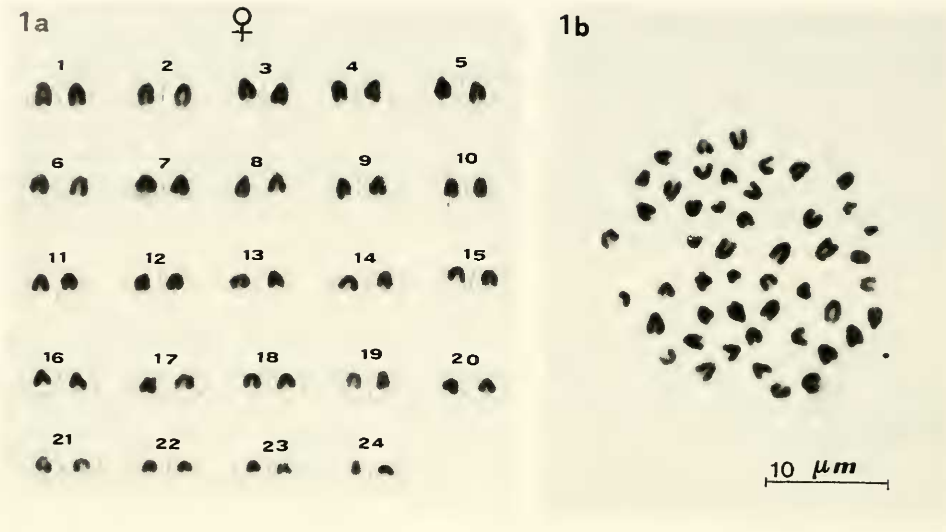
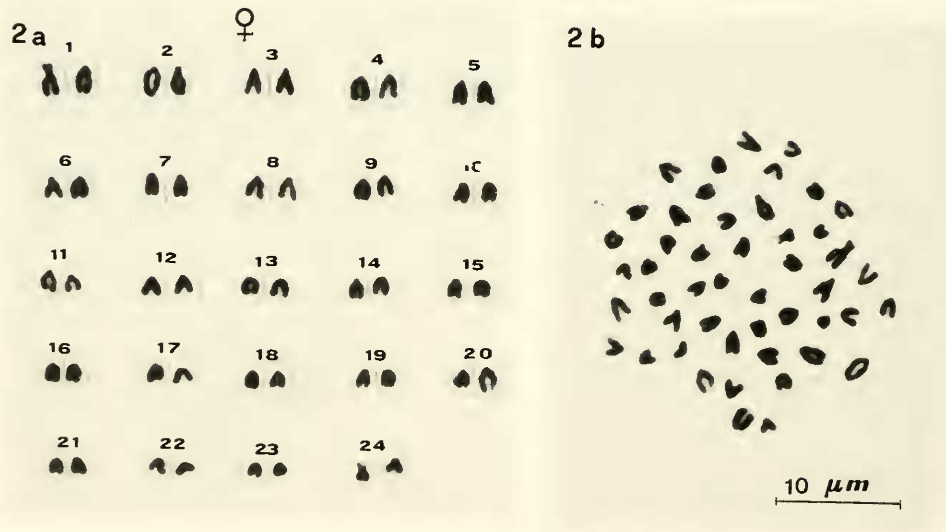




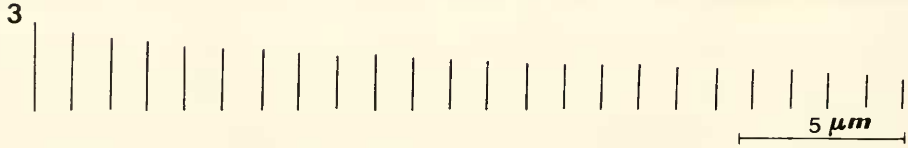


FIGURE 1a, b. Karyotype and metaphase plate of female *Gobius paganellus* (2n = 48).

FIGURE 2a, b. Karyotype and metaphase plate of female *Gobius paganellus* (2n = 48) with one subtelocentric chromosome.

FIGURE 3. Average karyotype obtained from nine metaphase plates of *G. paganellus*.

TABLE II

Mean length and arm ratio of the A-type chromosomes of nine metaphase plates (2n = 48) of Gobius paganellus *(specimens 1–7)*

Chromosome pairs	Mean length in microns ±S.D.	Arm ratio mean	Centromere position
1	2.67 ± 0.24	∞	A
2	2.31 ± 0.32	∞	A
3	2.18 ± 0.26	∞	A
4	2.07 ± 0.20	∞	A
5	2.01 ± 0.22	∞	A
6	1.95 ± 0.26	∞	A
7	1.88 ± 0.26	∞	A
8	1.79 ± 0.28	∞	A
9	1.72 ± 0.26	∞	A
10	1.64 ± 0.20	∞	A
11	1.62 ± 0.22	∞	A
12	1.58 ± 0.22	∞	A
13	1.53 ± 0.24	∞	A
14	1.48 ± 0.22	∞	A
15	1.43 ± 0.24	∞	A
16	1.43 ± 0.24	∞	A
17	1.39 ± 0.24	∞	A
18	1.35 ± 0.20	∞	A
19	1.30 ± 0.22	∞	A
20	1.22 ± 0.17	∞	A
21	1.17 ± 0.17	∞	A
22	1.10 ± 0.20	∞	A
23	0.95 ± 0.14	∞	A
24	0.84 ± 0.05	∞	A

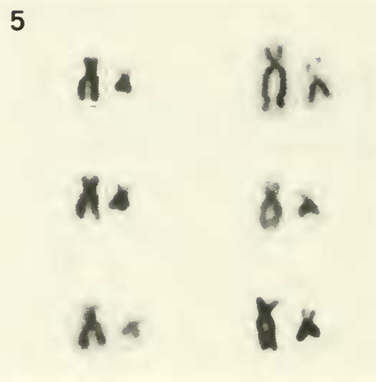
In some specimens with this karyotype a small acrocentric chromosome with satellites was identified with conventional staining (Fig. 4, see arrow). Figure 5 shows the sub-telocentric chromosome (first pair) and the small chromosome with its satellites (24th pair) obtained from different plates.

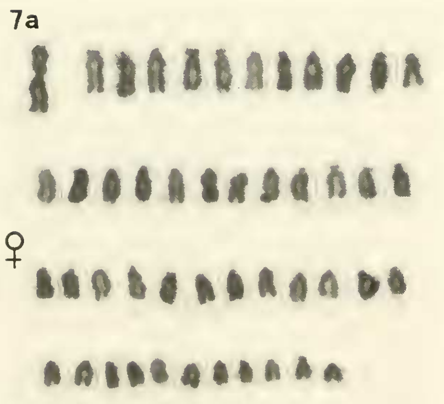
The other nineteen specimens studied differ from those showing an A-type complement not only for the chromosome number (Table I) but also for the presence of one or two bi-armed elements. Some of these specimens show the small satellited acrocentric chromosome.

Average sizes of bi-armed chromosomes were obtained from five different plates for each specimen studied and are reported in Table III. Analysis of average arm ratio in bi-armed chromosomes shows that they are two metacentric chromosomes of different size.

Of the nineteen specimens studied, one male (Fig. 6a, b), one female (Fig. 7a, b), and one sexually immature specimen with 2n = 47 showed MI, but four males (Fig. 8a, b) and four females (Fig. 9a, b) also with 2n = 47 showed element MII. Of the six specimens with 2n = 46, four females (Fig. 10a, b) showed two MII, one female (Fig. 11a, b) only one MII, and one male (Fig. 12a, b) both bi-armed chromosomes MI and MII. Two specimens of different sex (Fig. 13a, b; 14a, b) with 2n = 45 showed MI and MII.

Results summarized in Table IV show that the population of *Gobius paganellus* examined here possessed two fundamental numbers: NF = 47, and NF = 48.





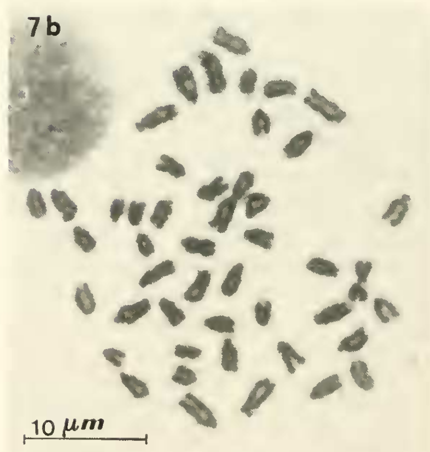


FIGURE 4. Metaphase plate of *G. paganellus.* (Arrow shows the small chromosome with its satellites.)

FIGURE 5. Sub-telocentric chromosome (first pair) and small chromosome (24th pair) obtained from different plates of *G. paganellus.*

FIGURE 6a, b. Alignment of somatic chromosomes and metaphase plate of male *G. paganellus* (2n = 47).

FIGURE 7a, b. Alignment of somatic chromosomes and metaphase plate of female *G. paganellus* (2n = 47).

TABLE III

Average length of bi-armed chromosomes from five metaphase plates for each non-A type specimen of Gobius paganellus *(MI = long bi-armed chromosome; MII = small bi-armed chromosome)*

			No bi-armed chromosome		Mean length in microns ±S.D.		Arm ratio mean	
Specimens	Sex	2n	MI	MII	MI	MII	MI	MII
8	♀	47	1		4.00 ± 0.92		1.34	
9	♂	47	1		3.95 ± 0.70		1.29	
10	?	47	1		5.05 ± 1.25		1.27	
11	♂	47		1		2.18 ± 0.26		1.26
12	♂	47		1		2.25 ± 0.60		1.39
13	♀	47		1		3.27 ± 0.67		1.32
14	♀	47		1		2.86 ± 0.38		1.35
15	♂	47		1		2.84 ± 0.30		1.24
16	♀	47		1		2.73 ± 0.43		1.26
17	♀	47		1		2.50 ± 0.45		1.29
18	♂	47		1		2.39 ± 0.26		1.25
19	♂	46	1	1	4.00 ± 1.17	2.27 ± 0.53	1.26	1.29
20	♀	46		2		2.64 ± 0.26		1.25
21	♀	46		2		2.64 ± 0.65		1.23
22	♀	46		2		2.36 ±0.35		1.34
23	♀	46		2		2.73 ± 0.80		1.31
24	♀	46		1		3.09 ± 0.59		1.27
25	♂	45	1	1	5.50 ± 1	3.06 ± 0.46	1.28	0.37
26	♀	45	1	1	4.59 ± 0.52	2.41 ± 0.26	1.24	1.26

DISCUSSION

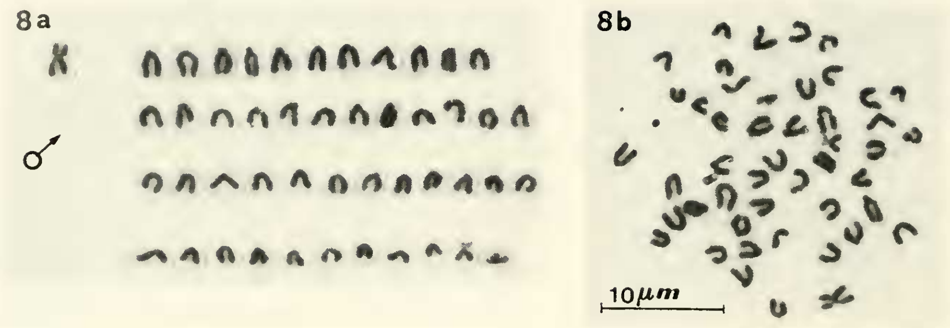
The present investigation was aimed at determining chromosome number in *Gobius paganellus;* modal numbers were found to be 2n = 45, 2n = 46, 2n = 47, and 2n = 48 (Table I). Figures with other than the modal number (aneuploid cells) found in our study were probably artifacts of preparation.

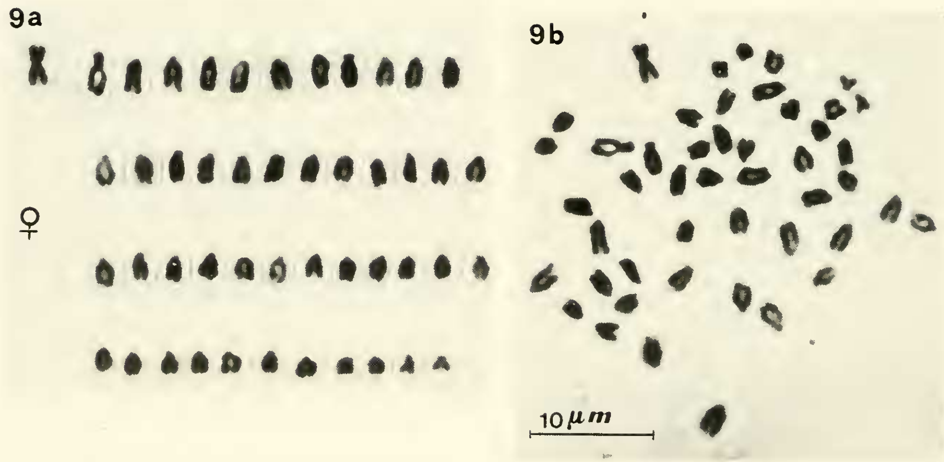
None of the above numbers agrees with 2n = 50, proposed by Colombera and Rasotto (1982) for this species. Values 2n = 45 and 2n = 46, however, confirm those reported by Cataudella *et al.* (1973) and Thode *et al.* (1983), respectively.

Comparison of chromosome sets observed by the latter authors and those reported in the present paper shows the following substantial differences: (1) Cataudella *et al.* (1973) established that in one female specimen of *Gobius paganellus* the diploid chromosome set 2n = 45 includes only one long metacentric chromosome. In our two specimens with 2n = 45, two bi-armed chromosomes (MI, and MII) (Table III) occurred. (2) Thode *et al.* (1983) reported the diploid value 2n = 46 (NF = 47 in males and NF = 48 in females) in this species and proposed a sex-determining mechanism XX/XY. Such a mechanism is not confirmed by the present research since specimens of different sex were found to have exactly the same karyotype (Table III; Fig. 6a, b; 7a, b; 8a, b; 9a, b; 13a, b; 14a, b)

Interestingly enough, only three species (over 50 species studied) of the Gobiidae show heteromorphic sex chromosomes (Nogusa, 1955; Subrahmanyam, 1969; Arai and Sawada, 1974).

The possibility that chromosome number may vary between different populations of the same species within this family was recently proposed by Colombera and Rasotto (1982). We found such variability in specimens from one popultion of *Gobius paganellus.* We found different diploid chromosome numbers as well as variations





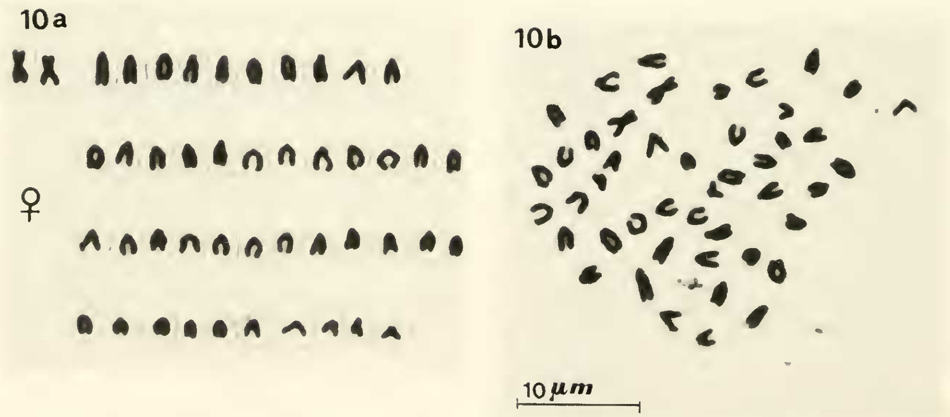
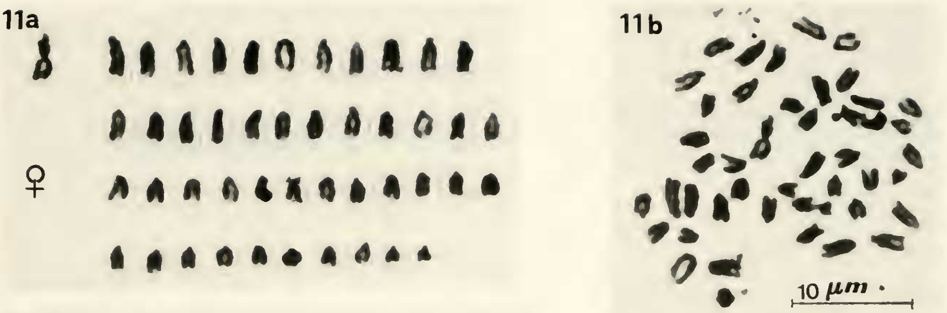
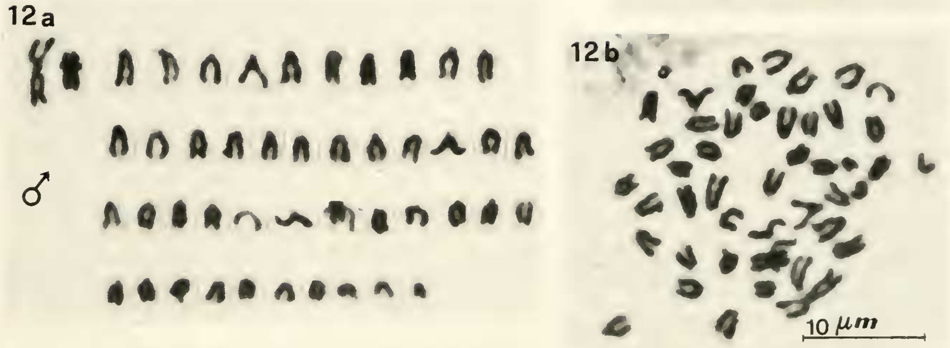


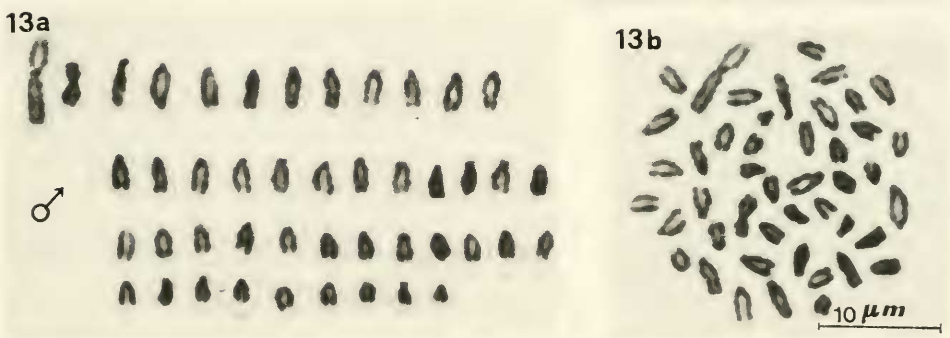
FIGURE 8a, b. Alignment of somatic chromosomes and metaphase plate of male *G. paganellus* (2n = 47).

FIGURE 9a, b. Alignment of somatic chromosomes and metaphase plate of female *G. paganellus* (2n = 47).

FIGURE 10a, b. Alignment of somatic chromosomes and metaphase plate of female *G. paganellus* (2n = 46).







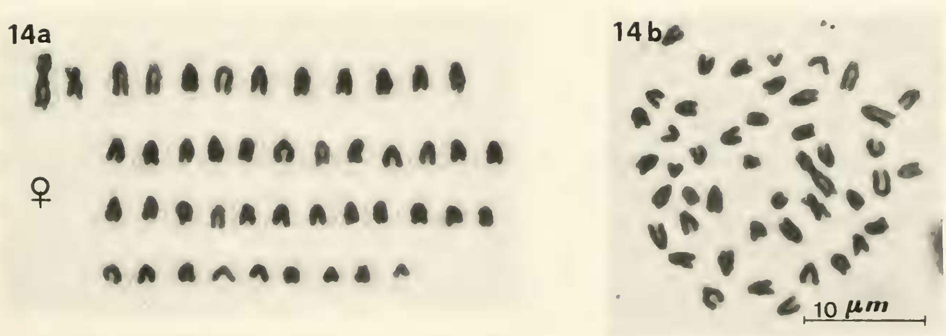


FIGURE 11a, b. Alignment of somatic chromosomes and metaphase plate of female *G. paganellus* (2n = 46).

FIGURE 12a, b. Alignment of somatic chromosomes and metaphase plate of male *G. paganellus* (2n = 46).

FIGURE 13a, b. Alignment of somatic chromosomes and metaphase plate of male *G. paganellus* (2n = 45).

FIGURE 14a, b. Alignment of somatic chromosomes and metaphase plate of female *G. paganellus* (2n = 45).

TABLE IV

Diploid numbers, chromosome morphology, and fundamental number (NF) in Gobius paganellus

Number of specimens	2n	Morphology			NF
		MI	MII	A + ST	
2	45	1	1	43	47
1	46	—	1	45	47
1	46	1	1	44	48
4	46	—	2	44	48
8	47	—	1	46	48
3	47	1	—	46	48
7	48	—	—	48	48
26					

in the chromosome arm number (NF) in this species. With respect to the latter, specimens studied in the present research had either NF = 48 or NF = 47, as shown in Table IV.

According to numerous authors (Ohno *et al.,* 1969; Ebeling and Chen, 1970; Ohno, 1970; Chen, 1971; Thode *et al.,* 1983) who maintain that 48 is the ancestral number in diploid fishes, we propose that the probable "wild type" karyotype of *Gobius paganellus* consists of 2n = 48 acrocentric chromosomes.

In specimens with 2n = 46 and 2n = 47 (NF = 48), the absence of two acrocentric chromosomes with respect to the A-type complement is regularly associated with the presence of one or the other of two metacentric chromosomes. It is clear therefore, that polymorphism for chromosome number is due to the presence of two independent Robertsonian fusions (MI and MII) in the population studied.

There is also a polymorphism for one sub-telocentric element in eight specimens which is probably due to a pericentric inversion.

Fishes with 2n = 46 to 2n = 48, but NF = 48 (Table IV) should be the result of random matings among balanced gametes produced in heterozygotes for each metacentric plus the two acrocentric homologs.

The NF = 47 fish observed here (Table IV), and NF = 50 fish found in other studies (Cataudella *et al.,* 1973) must be aneuploids. Then the variation of chromosome number as well as NF, must be the result of fusion of unbalanced gametes formed in either type of heterozygotes.

Robertson's translocation, pericentric inversion, and loss of chromosome segments are, in fact, considered to be chiefly responsible for chromosomal polymorphism in several species (White, 1969; Chen, 1971; Denton, 1973).

Finally, it should be noted that chromosome polymorphism observed in *Gobius paganellus* is not an isolated finding within the Pisces: it has also been found in the genus *Drascyllus* (Ojima and Kasciwagi, 1981), in the genus *Fundulus* (Chen, 1971; Black and Howell, 1978) and in the genus *Salmo* (Hartley and Horne, 1984 and authors quoted by them).

Examples of polymorphic species are reported in other animal groups (Ford *et al.,* 1957; Bianchi *et al.,* 1969; Lee and Zimmerman, 1969; authors quoted by White, 1969; Bantock and Cockayne, 1975).

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LITERATURE CITED

ARAI, R., AND Y. SAWADA. 1974. Chromosomes of Japanese gobioid fishes I. *Bull. Natl. Sci. Mus. Tokyo* **17**(2): 97–105.

ARAI, R., AND Y. SAWADA. 1975. Chromosomes of Japanese gobioid fishes III. *Bull. Natl. Sci. Mus. Tokyo* **1**(4): 225–239.

ARAI, R., I. KATSUYAMA, AND Y. SAWADA. 1974. Chromosomes of Japanese gobioid fishes II. *Bull. Natl. Sci. Mus. Tokyo* **17**(4): 269–279.

BANTOCK, C. R., AND W. C. COCKAYNE. 1975. Chromosomal polymorphism in *Nucella lapillus. Heredity* **34**: 231–245.

BIANCHI, N. O., J. CONTRERAS, AND F. N. DULOUT. 1969. Intraspecies autosomal polymorphism and chromosome replication in *Akodon molinae.* (Rodentia: Cricetidae). *Can. J. Genet. Cytol.* **11**: 233–242.

BINI, G. 1969. *Atlante dei Pesci delle Coste Italiane.* Vol. VII, pp. 109–110.

BLACK, A., AND M. W. HOWELL. 1978. A distinctive chromosomal race of the cyprinodontid fish, *Fundulus notatus,* from the upper Tombigbee River system of Alabama and Mississippi. *Copeia* **2**: 280–288.

CATAUDELLA, S., V. CIVITELLI, AND E. CAPANNA. 1973. The chromosomes of some Mediterranean teleosts: Scarpaenidae, Serranidae, Labridae, Blennidae, Gobiidae (Pisces Scarpaeniformes, Perciformes) *Boll. Zool.* **40**: 385–389.

CHEN, T. R. 1971. A comparative chromosome study of twenty killifish species of the genus *Fundulus* (Teleostei, Cyprinodontidae). *Chromosoma (Berl.)* **32**: 436–455.

CHEN, T. R., AND A. W. EBELING. 1971. Chromosomes of the goby fishes in the genus *Gillichthys. Copeia* **1**: 171–174.

CHIARELLI, A. B., AND E. CAPANNA. 1973. *Cytotaxonomy and Vertebrate Evolution.* Academic Press, London-New York.

COLOMBERA, D., AND M. RASOTTO. 1982. Chromosome studies in males of *Gobius niger jozo* (Padoa) and *Gobius paganellus* (Linneo) (Gobiidae, Osteichtyes). *Caryologia* **35**(2): 257–260.

DENTON, T. E. 1973. *Evolution of the Fish Karyotype. Fish Chromosoma Methodology.* Charles C. Thomas Publisher, Illinois. Pp. 129–148.

EBELING, A. W., AND T. R. CHEN. 1970. Heterogamety in teleostean fishes. *Trans. Am. Fish. Soc.* No. 1, **99**: 131–138.

FAGE, L. 1925. Rémarqués sur l'évolution des gobiidae accomagnees d'un synopsis des éspecies europeennes. *Arch. Zool. Exp. Gen.* **55**: 17–34.

FORD, C. E., J. L. HAMERTON, AND G. B. SHARMAN. 1957. Chromosome polymorphism in the common shrew. *Nature* **180**: 392–393.

HARTLEY, S. E., AND M. T. HORNE. 1984. Chromosome polymorphism and constitutive heterochromatin in the Atlantic salmon, *Salmo salar. Chromosoma (Berl.)* **89**: 377–380.

KHUDA-BUKHSH, A. R. 1978. Somatic chromosomes of an estuarine fish, *Trypauchen vagina* (Fam. Gobiidae) from Sagar Island, West Bengal, India. *Curr. Sci.* **47**(4): 137–138.

LEE, M. R., AND E. G. ZIMMERMAN. 1969. Robertsonian polymorphism in the cotton rat, *Sigmodon fulviventer. J. Mammalol.* **50**: 333–339.

LEVAN, A., K. FREDGA, AND A. A. SANDBERG. 1964. Nomenclature for centromeric position of chromosomes. *Hereditas* **52**: 201.

MANNA, G. K., AND R. PRASAD. 1974. Chromosome analysis in three species of fishes belonging to family Gobiidae. *Cytologia* **39**: 609–618.

NISHIKAWA, S., K. AMAOKA, AND K. NAKANISHI. 1974. A comparative study of chromosomes of twelve species of Gobioid Fishes in Japan. *Jpn. J. Ichthyol.* **21**(2): 61–71.

NOGUSA, S. 1955. Chromosome studies in Pisces IV. The chromosomes of *Mogrunda obscura* (Gobiidae), with evidence of male heterogamety. *Cytologia* **20**: 11–18.

OHNO, S. 1970. The enormous diversity in genome sizes of fish as a reflection of nature's extensive experiments with gene duplication. *Trans. Am. Fish. Soc.* **99**(1): 120–130.

OHNO, S., J. MURAMOTO, C. STENIUS, L. CHRISTIAN, W. A. KITTRELL, AND N. B. ATKIN. 1969. Microchromosomes in Holocephalian, chondrostean and holostean fishes. *Chromosoma (Berl.)* **26:** 35–40.

OJIMA, Y., AND E. KASHIWAGI. 1981. Chromosomal evolution associated with Robertsonian fusion in the genus *Drascyllus* (Chrominae, Pisces). *Proc. Jpn. Acad.* **57**(Ser. B.) 368–370.

SOLA, L., S. CATAUDELLA, AND E. CAPANNA. 1981. New developments in vertebrate cytotaxonomy. III. Karyology of bony fishes: a review. *Genetica* **54:** 285–328.

SUBRAHMANYAM, K. 1969. A karyotype study of the estuarine fish *Boleophtalmus boddaerti* (Pallas) with calcium treatment. *Curr. Sci.* **38**(18): 437–439.

THODE, G., J. CANO, AND M. C. ALVAREZ. 1983. A karyological study on four species of Mediterranean Gobiid fishes. *Cytologia* **48:** 131–138.

TORTONESE, E. 1975. *Fauna d'Italia. Osteichthyes—pesci ossei.* Calderini Publisher, Italia.

WHITE, M. J. D. 1969. Chromosomal rearrangements and speciation in animals. *Annu. Rev. Genet.* **3:** 75–98.